5.7.4.2 Chlorine

Freshwater acute (CMC) criterion = 19 µg/L
Freshwater chronic (CCC) criterion = 11 µg/L

Marine water acute (CMC) criterion = 13 µg/L
Marine water chronic (CCC) criterion = 7.5 µg/L

CAS ID: Elemental Chlorine 7782-50-5; CAS ID numbers for chemical forms of chlorine combined with other elements presented in Measures of Effect section

Chemical formula: Cl_2; chemical formula for chemical forms of chlorine combined with other elements presented in Measures of Effect section

Synonyms / Trade names: Chlorine dioxide, sodium hypochlorite, bleach, Clorox, HTH chlorine, calcium hypochlorite

Hatchery use: Three Washington hatcheries report using chlorine to disinfect effluent from isolation buildings that house fish from another watershed (to prevent disease spread from basin to basin). This use of chlorine could potentially result in its release to receiving waters where T&E species are present, although chlorine solutions are often neutralized with sodium thiosulfate before discharge into the environment. Two Washington hatcheries report spraying chlorine on dewatered raceways at the end of the season for disinfection purposes, then allow the chlorine solution to dry. It is not anticipated that any chlorine from its use on dewatered raceways would reach receiving waters where T&E species are present. None of the three hatcheries currently using chlorine discharge directly to estuarine or marine systems. Thus, under current use conditions, T&E species in marine waters are not exposed to chlorine releases from hatcheries.

Dried chlorine solutions on raceway walls (indeed on any surface exposed to outdoor ambient light) rapidly degrade so that the concentration of biologically active chlorine is reduced to zero. The chemistry of these reactions is described in detail in the environmental chemistry and fate of chlorine section later in this chapter.

The NPDES permit for Washington hatcheries contains effluent limitations set at the Washington chronic chlorine standards of 11 µg/L (discharges into freshwater) or 7.5 µg/L (discharges into estuarine or marine waters). It is these chlorine permit limits that are evaluated for risks to T&E species in this BE. Chlorine discharges at concentrations exceeding the chronic criteria will violate the NPDES permit limit for Washington hatcheries, and will not be allowed. Therefore, evaluation of the acute chlorine criteria is not germane to hatchery discharges in this BE.

5.7.4.2.1 Introduction

Unlike all other hatchery chemicals used in Washington and evaluated in this BE, chlorine has both acute and chronic water quality standards within both the fresh and marine waters of Washington State. The Washington chlorine standards (Ecology 2012) are identical to the EPA (1985) water quality criteria for chlorine. The chlorine standards do not apply to chloride ion concentrations in freshwater, for which both EPA and Washington State have separate chloride criteria and standards. The freshwater chloride standards are discussed in the Problem Formulation section of this BE.

The only previous biological evaluation of the chlorine water quality criteria of which EPA is aware was performed for proposed water quality standards of the Coeur d'Alene Tribe in Idaho (EPA 2013). The Coeur d'Alene Tribe chlorine standards, numerically the same as the Washington State chlorine standards, apply only to freshwaters where bull trout was the only threatened and endangered species present. The BE for the Coeur d'Alene Tribe water quality standards concluded that the proposed Tribal chlorine standard was not likely to adversely affect bull trout, a conclusion with which the USFWS concurred. No threatened or endangered species for which NMFS has trust responsibilities are present within waters subject to the Coeur d'Alene Tribe water quality standards, thus NMFS did not prepare an opinion on the Coeur d'Alene Tribe chlorine standards.

The current NPDES general permit for Washington hatcheries, as well as the new permit both have effluent limitations for chlorine set at the chronic criterion chlorine concentration of 11 μg/L and 7.5 μg/L for fresh and marine waters, respectively.

5.7.4.2.2 Problem Formulation

*Objective of the Biological Evaluation of the Chlorine Aquatic Life Criter*ia

The objective of this section of the BE is to determine whether an EPA approval of the proposed NPDES permit limit for chlorine, which is equivalent to the chronic chlorine national water quality criterion and Washington chronic chlorine standard is protective of T&E species.

Mechanism of Toxic Action of Chlorine

The toxic mechanism(s) of action of residual chlorine to aquatic life are not fully understood, but are likely related to the ability of chlorine to oxidize organic matter. Intracellular enzymes containing sulfhydryl groups are oxidized almost immediately by residual chlorine in both plants and animals. Due to the strength of the chemical bond formed between chlorine and proteins, enzyme activity is irreversibly terminated. This irreversible nature of chlorine reacting with enzymes likely explains the observed irreversible toxicity of chlorine to fish once equilibrium has been lost (Alabaster and Lloyd 1982).

In fish, gills are believed to be the primary site of toxic action of chlorine. This is based on multiple observations of damage to gill epithelium following exposure to chlorine. Cairns et al. (1975) concluded that the mode of toxic action of chlorine to fish is gill tissue damage combined with accumulation of mucus on the gills. The combination of physical damage to gill tissue and coating of gill tissue by mucus inhibits oxygen uptake, resulting in suffocation of the fish.

If the mechanism of toxic action proposed by Cairns et al. (1975) is correct, chlorine is one of the relatively few chemicals that does not require an internally bioaccumulated dose to elicit toxicity to aquatic life. The mechanism of toxic action of chlorine limits the exposure of both fully aquatic and aquatic-dependent species to chlorine, as it precludes exposure via the dietary ingestion exposure route. Ingestion via drinking water is an insignificant contaminant exposure pathway to freshwater fish, which are physiologically constrained from ingesting water because of their need to maintain a higher internal solute content than found in their external freshwater environment.

Conceptual Model of Chlorine Toxicity to T&E Species and their Prey

The reactivity of chlorine with other substances found in aquatic systems, combined with the volatility of chlorine gas limits both the concentration and residence time of chlorine in aquatic systems. Unlike most other chemicals discharged to aquatic systems, sediments do not serve as a sink for chlorine. Sediment is therefore not a medium by which aquatic species are exposed to chlorine. The combination of these factors also serves to limit the complete and significant exposure pathway of aquatic species to chlorine discharged to surface waters to direct contact, primarily with respiratory surfaces of aquatic species.

Consistent with the mode of toxic action for chlorine, dietary ingestion of chlorine is considered an insignificant exposure route for both T&E fully aquatic and aquatic-dependent species, as well as their prey in this BE. Therefore, the dietary ingestion exposure route will not be quantitatively evaluated for any species in this BE.

Measures of Exposure

As described in the methodology section, the expected environmental concentration (EEC) of chlorine is set at either 11 μg/L (freshwater) or 7.5 μg/L (estuarine or marine water). These values are the respective chronic criteria for chlorine in fresh and marine waters. They are also the proposed Washington hatcheries NPDES permit effluent discharge limits at 'end of pipe'.

Environmental Chemistry and Fate of Chlorine

Chlorine is a chemical element, atomic number 17, atomic weight 35.453. Except for minute amounts released to the atmosphere from volcanic eruptions, elemental chlorine is not found in a free state in nature due to its reactive nature. Elemental chlorine is a yellowish green gas under all conditions normally found in the environment except for extreme cold temperatures (boiling point = -34°C or -29°F). Elemental chlorine is most commonly produced by the chloralkali process, which is the electrolysis of sodium chloride dissolved in water. Electrolysis of brine produces diatomic or elemental chlorine (Cl_2), hydrogen gas and sodium hydroxide.

Use of chlorine in hatcheries for disease control purposes mimics its use in public health. Use of chlorine since the early 1900's as a disinfectant in both drinking water and sewage before it is discharged to surface water, with the concomitant reduction or elimination of many waterborne infectious diseases has been identified as one of the top ten advances in public health of the 20th century (CDC 1999).

The water chemistry of chlorine in freshwater is among the most complex of any contaminant evaluated in this BE. In addition to having a complex chemistry, there are multiple names in the literature for the same or similar combinations of chlorine chemical forms, necessitating this discussion of chlorine chemistry and terminology used in this BE.

The EPA aquatic life criteria for chlorine describes the toxicity of total residual chlorine (TRC), which is the combined concentration of different chemical forms of chlorine able to react with other substances, or which can interconvert among each other. Within the literature, TRC is generally synonymous with reactive chlorine (RC), combined residual chlorine (CRC), and total available chlorine (TAC).

Total residual chlorine includes free available chlorine (FAC; hypochlorous acid [HOCl] and the hypochlorite ion [OCl^-]; also referred to as free residual chlorine [FRC]) and combined available chlorine (CAC; organic and inorganic chloramines [NH_2Cl or monochloramine, $NHCl_2$ or dichloramine, and NCl_3 or nitrogen trichloride]). Chloramines are also often termed N-chloramides.

In ambient freshwater, the dominant reactive chlorine species are hypochlorous acid and its associated hypochlorite anion in waters with low ammonia or nitrogen concentrations. The hypochlorite anion is one of several compounds or anions that collectively are called chlorine oxides, the best known of which may be the perchlorate anion ($HClO_4^-$). Hypochlorous acid and its associated hypochlorite anion, along with chlorine dioxide (ClO_2) are by far the chlorine oxides most commonly utilized in water disinfection. Chlorine dioxide is also commonly used in the industrial bleaching of wood pulp.

Like elemental chlorine, chlorine dioxide is also a gas at temperatures found in the environment. Rather than hydrolyzing in water as chlorine does, chlorine dioxide forms a true solution in water under typical surface water conditions. Chlorine dioxide is volatile and is easily lost from water. Chlorine dioxide is a powerful oxidant but unlike chlorine, does not readily combine with ammonia to form chloramines. Chlorine dioxide also does not form trihalomethanes such as chloroform. Due to its reactive nature, chlorine dioxide is produced on-site at locations where it is used as a disinfectant.

Monochloramine can be a dominant chemical form if sufficient nitrogen, particularly in the form of ammonia/ammonium ion is present in surface water. Di- and trichloramines are only formed in water at $pH < 6$ and when the $Cl_2:NH_3$ is at least 5:1 (Hankin 2001). Free chlorine gas (Cl_2) becomes the dominant chemical form only in low organic content waters with a $pH < 2$. Chlorine can also react with naturally occurring organic matter in water to form a number of disinfection byproducts, including trihalomethane compounds such as chloroform.

The initial chemical reaction when Cl_2 is added to surface water is one of hydrolysis (EPA 1976):

$$Cl_2 + H_2O \rightarrow HOCl + H^+ + Cl^-$$

Hypochlorous acid (HOCl) is a weak acid, and undergoes a pH dependent dissociation:

$$HOCl \leftrightarrow H^+ + OCl^-$$

The release of hydrogen ions from hydrolysis of Cl_2 and the dissociation of hypochlorous acid are the reasons chlorination of surface water tends to reduce the pH of the water. The ratio of HOCl to OCl^- is pH dependent, with 96% HOCl present at pH 6, 75% HOCl at pH 7, 22% HOCl at pH 8, and only 3% HOCl at pH 9. The proportion of HOCl present in water is significant, as HOCl is the chemical form most effective as a disinfectant (Shannon et al. 2008).

Analytical determination of the various chemical species within TRC is generally not performed, and is generally not feasible at the low µg/L concentrations of toxicological relevance in surface waters. This is the reason the EPA aquatic life criteria are expressed in terms of TRC, not as criteria for the individual chemical forms comprising TRC.

Without continuous addition of chlorine to water, TRC concentrations in water can be quickly reduced through several chemical, physical and biological processes. In addition to the chemical reactions in the water column described above, these processes include volatilization, photodegradation, adsorption on solids, and reactions with aquatic life.

Degradation rates of chlorine species in natural waters are generally rapid, ranging between seconds and hours. The half life of chlorine gas (Cl_2) in surface water has been reported as 0.005 second (EPA 1994). Cooper et al. (2007) have performed a number of photodegradation half life studies with HOCl / OCl^- mixtures under various pH values and water depths, and at several dissolved organic matter concentrations. The light intensity used was based on that at solar noon in both summer and winter at the latitude of Miami, Florida (24° N). In distilled water, the photodegradation half life of a HOCl / OCl^- mixture ranged between 41 minutes at pH 5.0 to 17 minutes at pH 7.0 to six minutes at pH 12.0. Half lives of a HOCl / OCl^- mixture were shortest in waters exposed to higher light intensity (i.e. summer light intensities), in waters with the lowest dissolved organic matter concentrations, and in waters of the shallowest depths. Shortest half lives of just over nine minutes occurred under conditions of summer light intensity in surface water at 0 meters depth and with dissolved organic matter concentrations of either 0.53 or 17.6 mg C/L. The only half lives longer than 10 hours observed by Cooper et al. (2007) occurred under conditions of water with a depth $\geq$ 1 meter with a dissolved organic matter concentration of 17.6 mg C/L under either summer or winter light intensity. In water containing 0.53 mg C/L dissolved organic matter and with depth $\leq$ 5 meters, all HOCl / OCl^- mixture half lives were 5.85 hours or shorter under all light intensities tested.

Historically at fish hatcheries, sodium thiosulfate has been used to neutralize and remove residual chlorine from water in which fish are eventually to be held. The reaction of sodium thiosulfate with chlorine produces sodium chloride as the end product of the residual chlorine, with the thiosulfate being converted to sodium tetrathionate, as follows:

$$Cl_2 + 2Na_2S_2O_3x5H_2O \rightarrow Na_2S_4O_6 + 2NaCl + 10\ H_2O$$

The stoichiometric ratio of sodium thiosulfate to residual chlorine to completely neutralize the chlorine without the addition of excess sodium thiosulfate is 6.99:1 (i.e. 6.99 mg/L sodium thiosulfate pentahydrate neutralizes 1 mg/L chlorine).

Sodium thiosulfate also neutralizes hypochlorous acid and monochloramines according to the following reactions:

$$2Na_2S_2O_3 + HOCl \rightarrow Na_2S_4O_6 + NaCl + NaOH$$

$$NH_2Cl + 2Na_2S_2O_3x5H_2O \rightarrow Na_2S_4O_6 + 2NaOH + NH_3 + HCl + 3H_2O$$

Several reactions occur in chlorine solutions which are sprayed on hatchery surfaces such as raceways, then allowed to dry, that reduce chlorine to non-toxic chemical forms. The two most common reactions both involve transformation into sodium chloride, with either sodium chlorate ($NaClO_3$) or elemental oxygen as byproducts. These reactions are illustrated using sodium hypochlorite as the chlorine solution sprayed onto surfaces, as follows:

$$3NaOCl \rightarrow 2NaCl + NaClO_3 \text{ (chlorate formation)}$$

$$2NaOCl \rightarrow 2NaCl + O_2 \text{ (elemental oxygen formation)}$$

Warmer temperatures, higher hypochlorite concentrations and higher ionic strength (i.e. the concentration of salts in water) all serve to increase the reaction rate of the breakdown of hypochlorite solutions to sodium chloride and either sodium chlorate or elemental oxygen. The conversion of hypochlorite to chlorate is the more common of the two reactions. The formation of sodium chloride and sodium chlorate accounts for the white powder often observed on surfaces after hypochlorite solutions have dried. Sunlight also speeds up the decomposition of hypochlorite solutions through the process of photolysis.

$$2OCl^- + \text{ultraviolet light} \rightarrow 2Cl^- + O_2 \text{ (photolysis)}$$

$$2NaOCl + \text{ultraviolet light} \rightarrow 2NaCl + O_2 \text{ (photolysis)}$$

In water with a pH of 8.0, the half-life of sodium hypochlorite undergoing the above photolysis reactions is 12 minutes (Oltchim 2011). The photolysis half-life of chlorine in water varies with pH, chlorine/hypochlorite concentration, light intensity and water temperature, with pH having the largest effect. Forsyth (2012) evaluated the half-life of chlorine during photolysis under a range of pH (6, 7 and 8), water temperatures (10° and 25°C), light intensities up to full natural sunlight intensity during May at latitude 47°N, and chlorine chemical forms, and observed a range of half-lives between 9 – 96 minutes. The half-life of chlorine under photolysis gets longer as the pH becomes more acidic. Metal cations in water, including iron, nickel, cobalt and copper also catalyze the breakdown of hypochlorite anion (OCl^-) to chloride anion (Cl^-) and oxygen. Allowing chlorine solutions to completely dry on surfaces, particularly outdoor surfaces for a 24 hour period before they are rinsed or refilled with water should reduce the concentration of biologically active chlorine forms to non-toxic levels.

In marine and estuarine waters, the chemistry of chlorine is, if anything, even more complex than it is in freshwater. Full strength (35‰) seawater contains roughly 70 mg/L bromide ion, mostly in the form of sodium bromide. This is substantially higher than the EPA chronic chlorine criterion for saltwater of 7.5 µg/L. But because elemental chlorine (Cl_2) has a higher standard reduction potential (i.e. is a stronger oxidant) than does elemental bromine (Br_2), chlorine can displace bromine from sodium bromide via the following reaction:

$$2\ NaBr + Cl_2 \rightarrow 2NaCl + Br_2$$

Other chemical forms of chlorine, including hypochlorous acid can also rapidly react with bromide in seawater to form a series of brominated compounds (Singleton 1989). If ammonia is present, the brominated compounds can form a series of bromamines analogous to the chloramines formed in freshwater. Dibromamine is the most commonly formed bromamine in sea water of pH = 8. Several of the more important chlorine and bromine reactions in sea water are shown below.

$$\mathrm{HOCl} + \mathrm{Br^-} \leftrightarrow \mathrm{HOBr} + \mathrm{Cl^-} \quad \text{(hypochlorous acid converts to hypobromous acid)}$$
$$\mathrm{OCl^-} + \mathrm{Br^-} \leftrightarrow \mathrm{OBr^-} + \mathrm{Cl^-} \quad \text{(hypochlorite converts to hypobromite)}$$
$$\mathrm{HOBr} + \mathrm{NH_3} \leftrightarrow \mathrm{NH_2Br} + \mathrm{H_2O} \quad \text{(monobromamine formation)}$$
$$\mathrm{HOBr} + \mathrm{NH_2Br} \leftrightarrow \mathrm{NHBr_2} + \mathrm{H_2O} \quad \text{(dibromamine formation)}$$

These reactions are important to understand the disinfecting ability of chlorine in sea water. The rapid formation of brominated compounds in sea water after the addition of chlorine means in practice much of the disinfecting capacity of chlorine in sea water is actually due to bromine compounds, not chlorine compounds. As acknowledgement of the role of bromine in disinfection in marine and estuarine systems, the term ‘chlorine produced oxidants’ is often used to describe the sum of the concentrations of all oxidative chemical forms of chlorine and bromine in saltwater. The standard analytical methods (most commonly amperometric titration) used to measure total residual chlorine in freshwater also detect the various chemical forms of bromine in saltwater. However, due to the presence of both chlorinated and brominated compounds in saltwater with disinfecting properties, results of the analysis for chlorine in saltwater are often expressed in units of µg/L chlorine produced oxidants, not µg/L total residual chlorine as is the case in freshwater.

The short persistence of chlorine in water relative to the duration of standard toxicity tests with fish and invertebrates has direct bearing on the experimental design of toxicity studies useable to evaluate chlorine toxicity to T&E fish species. In order to maintain a consistent concentration of chlorine in laboratory toxicity tests, flow through studies where chlorine concentrations are constantly replenished are needed. EPA’s water quality criteria are designed to apply in situations of continuous exposure to a contaminant. They are not designed to be applied in situations of intermittent contaminant exposure. Much of the available aquatic toxicity data for chlorine describes information generated during either very short term studies (three hours or shorter), from exposure to chlorinated sewage effluent (an unacceptable dilution water) or from intermittent exposures. These short term and intermittent studies are not suited for EPA water quality criteria development or evaluation of effects on threatened or endangered species, as they are not representative of effects from continuous exposure to chlorine. The chlorine effects determination within this BE are therefore based only on continuous flow through exposures of acceptable duration (96 hours for acute mortality studies with fish).

Measures of Ecosystem and Receptor Characteristics

Section ??? describes the range, critical habitat, life history, population trends and status of the T&E species evaluated in this toxicity assessment.

Measures of Effect

To characterize ecological effects, it must first be verified that the stressor elicits adverse effects on ecological entities of interest. Once verified, the adverse effects elicited by the stressor are described, and then evaluated in terms of how the magnitude of adverse effect changes as the concentration of the stressor changes. Finally, it is confirmed that the observed effects are consistent with the environmental values to be protected as described in the assessment endpoints, as well as confirming that the exposure conditions under which the observed adverse effects occur are consistent with the conceptual model.

This chlorine toxicity assessment, the primary focus of this Measures of Effect section, is based completely on existing information. The toxicity data for aquatic species is that presented in the EPA (1985) water quality criteria document for chlorine has been augmented by 2012 and 2013 EPA literature searches for additional toxicity information published in the literature subsequent to the publication of the EPA (1985) chlorine criteria document. The toxicity assessment infers or extrapolates chlorine effects on T&E fish species and their prey from this existing data.

All measures of effect in this toxicity assessment are laboratory toxicity tests where empirically measured chlorine concentrations in water were associated with adverse effects on survival, reproduction or growth of aquatic species. Mixture studies where chlorine was part of a mixture of contaminants to which a test species was exposed are not included in the measures of effect data, as it is generally not possible to attribute the proportion of the response due to chlorine.

Specifically, mixture studies where aquatic species were exposed to sewage or wastewater disinfected with chlorine were excluded as a primary line of evidence in this BE. This exclusion is because there is no quantitative method for separating the adverse effects of other contaminants in sewage from the adverse effects of chlorine. Unfortunately, a review of the studies used to derive the 1985 EPA aquatic life criteria for chlorine found that a number of the toxicity studies used to derive the criteria were performed on treated wastewater. The publication of the 1985 EPA chlorine criteria document predates the Stephan et al. (1985) guidance document which contains the procedures, including the data acceptability requirements of toxicity literature, currently used to derive EPA's water quality criteria. If the data acceptability requirements of Stephan et al. (1985) had been employed in the 1985 EPA chlorine criteria document, a number of the studies used to derive the chlorine criteria would have been excluded from criteria derivation.

The EPA 1985 chlorine criteria document is the basis for the chlorine effluent permit limit in the Washington hatcheries general NPDES permit. As such, we have chosen to evaluate the studies in the EPA 1985 chlorine criteria document using a line of evidence not employed for any other chemical in this BE. The ranked genus mean acute values in Table 3 of the EPA 1985 chlorine criteria document include species mean acute values for two freshwater T&E species under evaluation in this BE: coho salmon and steelhead (rainbow trout).

For marine waters, the 1985 EPA criteria document includes a species mean acute value for coho salmon of acceptable data quality (Thatcher 1978). The primary line of evidence in this evaluation of chlorine is the use of high quality acute toxicity data with the Interspecies Correlation Estimation (ICE) model and with acute-chronic ratio (ACR) for chlorine to calculate chronic no effect concentrations. With the exception of the saltwater coho salmon study of Thatcher (1978), all other evaluations in this section have been performed with ICE models where acute LC_{50} data with non-T&E salmonid species in Washington has been used as the input into the ICE model.

We have also for the purposes of the evaluation of chlorine in this BE assumed that all of the acute toxicity data in the EPA 1985 chlorine criteria for prey species of the T&E species met current data quality requirements. This assumption allowed us to convert species mean acute values from the criteria document into chronic no effect concentrations using an acute-chronic ratio. Although not based on as high a quality literature information as desired for this BE, the approach used to evaluate information from the 1985 EPA chlorine criteria document provides a secondary line of evidence in the toxicity assessment and risk characterization of chlorine. This secondary line of evidence provides an additional level of support for our conclusions regarding the protectiveness of the chlorine effluent limit in the Washington hatcheries NPDES general permit.

The three sources of measures of effect are 1.) The acute and chronic toxicity data for aquatic species in the EPA (1985) Ambient Water Quality Criteria for Chlorine, specifically Tables 1 and 2 (empirical acute and chronic toxicity, respectively) and 3 (empirical rank ordered genus and species mean acute toxicity data) from the chlorine criteria document; 2.) The additional toxicity data identified by EPA during its 2012 literature review on chlorine toxicity, and; 3.) A supplemental EPA 2013 literature review that searched specifically for toxicity information on chloramines and other chlorine chemical forms not searched for during the EPA 2012 literature review. The 2012 and 2013 literature reviews were originally performed for the Coeur d'Alene Tribe BE for their water quality standards. The 2013 EPA literature review in ECOTOX searched for all freshwater animal toxicity data for the following chlorine chemical forms listed in Table 5.???, an expanded list from the search performed in 2012.

Table 5.??? Chemicals for which Aquatic Toxicity Data Searches were Performed in ECOTOX.

Chemical	Chemical Abstracts Service ID
Chlorine (same CAS ID as TRC)	7782-50-5
Chlorine dioxide	10049-00-4
Monochloramine	10599-90-3
Dichloramine	3400-09-7
Trichloramine (nitrogen trichloride)	10025-85-1
Hypochlorous acid	7790-92-3
Hypochlorite anion	14380-61-1
Sodium hypochlorite	7681-52-9

Chlorine dioxide is reported as chlorine oxide in the ECOTOX output. Monochloramine is reported as chloramine in the ECOTOX output. Sodium hypochlorite is reported as hypochlorous acid, sodium salt (1:1) in the ECOTOX output.

No additional chronic toxicity data meeting current EPA data quality criteria requirements were found in addition to those already identified in Table 2 of the EPA (1985) chlorine water quality criteria document. Division of an LC_{50} by an acute-chronic ratio provides an estimate of a chronic no effect concentration in the absence of empirical chronic toxicity data for aquatic species. This is based on the standard ACR definition EPA historically has used, as described in Raimondo et al. (2007). "The ACR is calculated as the ratio of the median lethal concentration (LC_{50}) and a chronic no-observed-effect concentration (NOEC) or the maximum acceptable toxicant concentration (MATC). The MATC is the geometric mean of the NOEC and the lowest-observed-effect concentration (LOEC) determined from growth, reproduction, or survival endpoints."

Lines of Evidence

Information derived from different sources or by different techniques that can be used to describe and interpret risk estimates are called lines of evidence in ecological risk assessments. Sometimes more than one line of evidence is needed to reasonably demonstrate that stressors are likely to cause adverse effects on the assessment endpoint. This situation arises when either the amount of information available for a line of evidence is limited, or if substantial uncertainties exist regarding the information to be used in risk characterization. If multiple lines of evidence are evaluated and some lines of evidence conflict with others, professional judgment is needed to determine which data should be considered more reliable or relevant to the questions.

Once there is agreement on which lines of evidence are required to answer questions concerning the assessment endpoint, the measures of effect by which the risk hypotheses will be examined can be selected.

Empirical Toxicity Data Line of Evidence

Unfortunately, there are no empirical acute or chronic chlorine toxicity data for any of the freshwater T&E species under evaluation that meet current EPA data quality requirements for use in derivation of EPA water quality criteria. The only freshwater salmonid studies with chlorine that meet current EPA data quality requirements are a series of LC_{50} tests with brook trout (*Salvelinus fontinalis*) by Thatcher et al. (1976), and several LC_{50} tests with brook trout and cutthroat trout (*Oncorhynchus clarki*) performed by Larson et al. (1978). These two studies are the sources of the acute LC_{50} data used with the Interspecies Correlation Estimation (ICE) line of evidence described in the next section.

For marine systems, a chlorine acute LC_{50} study of acceptable data quality was performed by Thatcher (1978) on coho salmon. The LC_{50} from this study was used directly with the chlorine ACR to derive a chronic NOEC for coho salmon in marine systems. The coho salmon LC_{50} from Thatcher (1978) was used with the ICE model to estimate chlorine toxicity to the remaining salmonid species in marine waters. No chronic toxicity studies of acceptable data quality were identified for any T&E species under evaluation in the marine waters of Washington.

As described in the methodology, once an acceptable acute LC_{50} is identified for a T&E species with an existing water quality criterion, it is divided by the acute-chronic ratio (ACR) for that chemical to convert an acute LC_{50} into a chronic NOEC concentration.

Interspecies Correlation Estimation (ICE) Methodology Line of Evidence

It is impractical for toxicologists to perform laboratory toxicity studies on all aquatic species present in North America with all chemicals to which they are exposed in the environment. This is particularly true for ESA listed species, whose rarity or limited distribution in the environment generally precludes their use as test organisms in aquatic toxicology, except for limited research purposes. ICE models are statistical regressions that permit estimations of LC_{50}s to be made for a species or higher taxa (genus, family) having no measured acute toxicity information from a species for which five or more LC_{50}s have been measured. The detailed description of how ICE models were developed and their use to estimate LC_{50}s for taxa for which no toxicity information is available is given in Raimondo et al. (2013).

ICE models between two taxa are linear regressions of the form shown in Equation 5.???

Equation 5.2: $$\log_{10} X_2 = a + (b \times [\log_{10} X_1])$$

Where: X_1 is a measured LC_{50} value for an aquatic species (e.g. coho salmon, Daphnia magna)
X_2 is the predicted LC_{50} value for the taxa (species, genus or family) without toxicity data

The current version of ICE, called WebICE, is freely available from EPA on the Internet at: http://www.epa.gov/ceampubl/fchain/webice/

Based on the current data quality requirements for literature to be used in the derivation of EPA aquatic life criteria, a study of chlorine toxicity to brook trout by Thatcher et al. (1976), and a study of chlorine toxicity to brook trout and cutthroat trout by Larson et al. (1978) are the only freshwater studies with a salmonid that meets present day data quality requirements. The endangered species module of WebICE contains regressions between either brook trout or cutthroat trout and all other T&E species of the family Salmonidae under evaluation in this BE. The Thatcher et al. (1976) brook trout study was therefore used with WebICE to generate the regressions used to estimate LC_{50} values for bull trout, while the Larson et al. (1978) cutthroat trout results were used with WebICE to generate the regressions used to estimate LC_{50} values for all of the T&E Oncorhynchus species in freshwater under evaluation in this BE. The lower 95% confidence interval of the surrogate species empirically measured LC_{50} is calculated by the ICE model. If the study that is the source of the LC_{50} for the surrogate species does not report a lower 95% confidence interval, the LC_{50} itself is used as the input into ICE.

The ICE calculated lower 95% confidence interval of the LC_{50} for the T&E species of interest (derived from the empirical LC_{50} of a surrogate species) is then divided by the chlorine acute-chronic ratio (ACR) of 3.345, as presented in the EPA (1985) chlorine criteria document. The quotient resulting from dividing the LC_{50} by the ACR is the chlorine chronic no effect concentration (chronic NOEC) for the T&E species of interest. The risk characterization portion of this assessment compares the chronic NOEC for each T&E species to the chronic chlorine criterion to determine whether the NPDES permit limit for chlorine is protective of T&E species.

A second study by Thatcher (1978) contains 96 hour LC_{50} results in saltwater meeting current EPA data quality requirements for one of the T&E salmonid species under evaluation in this BE: coho salmon. The procedures described in the previous paragraph for relating a brook trout freshwater 96 hour LC_{50} to a chronic NOEC were used with the saltwater coho salmon 96 hour LC_{50} to generate chronic NOECs for the remaining T&E salmonid species in saltwater.

Species Mean Acute Values (SMAVs) from the 1985 EPA Ambient Water Quality for Chlorine – 1984 Line of Evidence

The EPA (1985) chlorine criteria document (the title of the chlorine criteria document indicates it was completed in 1984, but was not released until January 1985) calculated acute criteria for freshwater species from a species sensitivity distribution containing LC_{50} data from 28 different genera. Two of the freshwater T&E species under evaluation in this BE were included in the criteria derivation: coho salmon and rainbow trout (steelhead). At the time the criteria were published, rainbow trout were considered to be in a separate genus (Salmo) from coho salmon (Oncorhynchus), although today taxonomists consider both species to be in the genus Oncorhynchus. Cutthroat trout were also considered to be a Salmo species in the EPA chlorine criteria document, but are also currently considered to be members of the genus Oncorhynchus.

Among the 28 freshwater genera, coho were the 6th most sensitive to chlorine, rainbow trout and cutthroat trout (both considered as Salmo in the 1985 criteria document) the fourth most sensitive. The species mean acute values are available from the EPA (1985) chlorine criteria document. They are listed as 74.79 µg/L for coho, and 61.92 µg/L for rainbow trout. Within EPA water quality criteria documents, toxicity data when possible are carried to four significant digits, while the criteria values themselves are only reported to two significant digits.

A number of the studies used in the EPA (1985) chlorine criteria document to derive species mean acute values or genus mean acute values during the derivation of the acute chlorine criterion for freshwater do not meet current EPA data quality requirements. The studies not meeting current data quality requirements and the reason they do not meet the requirements are shown in Table ???

Table ??? Data quality rationale for excluding chlorine acute toxicity studies from the EPA (1985) chlorine criteria document as a primary line of evidence in this BE

Reference	Species	Data Quality Requirement Not Met
Lamperti 1976	Coho salmon	Control response not reported
Arthur et al. 1975	Coho salmon Brook trout	Dilution water quality not acceptable (fish exposed to chlorinated sewage effluent)

Ward et al. 1976	Coho salmon Rainbow trout Lake trout	Dilution water quality not acceptable (fish exposed to chlorinated sewage effluent)
Ward and DeGraeve 1978	Coho salmon Rainbow trout Lake trout	Dilution water quality not acceptable (fish exposed to chlorinated sewage effluent)
Rosenberger 1972	Coho salmon	Inappropriate test endpoint and duration (LT_{50} instead of LC_{50})
Merkens 1958	Rainbow trout	Exposure duration too short (2 hr.)
Wolf et al. 1975	Rainbow trout	Fish exposed to combination of thermal shock and chlorine
Buckley et al. 1976	Coho salmon (salt water)	Dilution water quality not acceptable (fish exposed to chlorinated sewage effluent)

As an additional, but secondary line of evidence in this BE, EPA has assumed that the fish studies in Table ??? meet EPA data quality requirements, and the chronic NOEC values derived from these studies for rainbow trout and coho salmon are compared to the freshwater chronic chlorine criteria in risk characterization. The freshwater chronic chlorine criterion (11 µg/L) is equivalent to the effluent discharge permit limit in the Washington hatcheries NPDES permit.

5.7.4.2.4 Risk Characterization

Risk characterization is the final phase of ecological risk assessment. It combines and integrates the products of the problem formulation and analysis phases to estimate and describe any identified adverse ecological effects related to the assessment endpoints. The relationships between stressors, effects, and ecological entities are used to reach conclusions regarding the occurrence of exposure and the adversity of existing or anticipated effects.

After estimating the risk, risk estimates are described in the context of the significance of any adverse effects and lines of evidence supporting their likelihood. Finally, the uncertainties of the risk assessment are described, followed by the conclusions and determinations of the risk characterization.

The approaches used in this risk characterization to assess chlorine toxicity to T&E species and their prey are summarized in Table 5.???

Table 5.???. Summary of Assessment Endpoints, Measures of Effect and Lines of Evidence Used in Toxicity Assessment of Chlorine.

Assessment Endpoint	Measures of Effect	Lines of Evidence

Survival, reproduction and growth of threatened and endangered species	For chronic effects: calculated chronic NOEC (no empirical chlorine NOEC data exists for any T&E species under evaluation)	Empirical high quality LC_{50} data for T&E species divided by acute-chronic ratio (ACR) to derive chronic NOEC
		Interspecies Correlation Estimation (ICE) model at species, genus or family level to estimate acute LC_{50} for T&E species without empirical toxicity data, which is then divided by the ACR to derive chronic NOEC
		For T&E species with empirical acute LC_{50} data that does not meet current EPA acceptable data quality criteria for water quality criteria derivation, assume such data does meet data quality criteria, then divide the acute LC_{50} by the ACR to derive chronic NOEC
	For effects on prey species: LC_{50}, EC_{50}, EC_{20}, NOEC, LOEC, calculated MATC, calculated acute and chronic EC_A	Comparison of acute and chronic EC_A for prey species to acute and chronic water quality criteria
	For multiple routes of exposure:	Not evaluated, bioaccumulated dose of chlorine not required to elicit toxicity, dietary ingestion is an incomplete or insignificant exposure pathway for aquatic species

Chronic Freshwater Chlorine Criterion

Empirical Data Line of Evidence

No empirical data are available that describe either the acute or chronic exposure responses of any of the T&E species under evaluation to chlorine in freshwater. Therefore, no risk characterizations have been made for any T&E species in freshwater based on direct measurements of chlorine toxicity to the T&E species.

Interspecies Correlation Estimation (ICE) Line of Evidence

Because of the complete absence of high quality empirical freshwater acute or chronic toxicity data for any T&E species under evaluation, high quality toxicity data from surrogate species has been used with the ICE model (Raimondo et al. 2013) to estimate chlorine toxicity to T&E species in freshwater. As discussed in the toxicity assessment, no high quality chronic toxicity data exists for any species which could be used as a surrogate species for a T&E species under evaluation.

The EPA (1985) chlorine criteria document and the 2012 and 2013 ECOTOX searches completed by EPA all identified the studies of Thatcher et al. (1976), which reported the effects of temperature changes on chlorine toxicity to juvenile brook trout (*Salvelinus fontinalis*), and that of Larson et al. (1978) with cutthroat trout (*Oncorhynchus clarkii*) as containing high quality 96 hour LC_{50} acute toxicity that can be used with ICE to estimate chlorine toxicity to all of the T&E salmonid species under evaluation.

Brook trout are the same genus as are bull trout (*Salvelinus confluentus*), and thus are expected to have similar sensitivity to contaminants as do bull trout. Brook trout, a species in the genus *Salvelinus* known to hybridize with bull trout is used as a surrogate species for bull trout in the ICE model as the starting point to derive a chronic NOEC for bull trout. Cutthroat trout are the same genus (*Oncorhynchus*) as the remaining five T&E species under evaluation (Chinook, chum, coho and sockeye salmon, steelhead). Cutthroat trout is used as the surrogate species in ICE for all freshwater T&E species of the genus *Oncorhynchus*. The rationale for these choices is shown in Table ??? (ICE predictions for chlorine – freshwater T&E species.xlsx, the Excel file is too wide to merge into the text) and the following discussion.

Within the Thatcher et al. (1976) study, six 96-hr LC_{50} studies performed at either 10°C or 15°C provide suitably high quality data that can be used to evaluate TRC toxicity to brook trout. LC_{50} values for the four tests run at 10°C and the two tests run at 15°C ranged between 131 – 179 µg/L. Temperature had no statistically distinguishable effect on the six LC_{50} values, so they were pooled to calculate a geometric mean 96-hr LC_{50} of 152 µg/L, with a 95% lower confidence limit of the mean LC_{50} of 136 µg/L.

Similarly, Larson et al. (1978) generated five 96 hour LC_{50} values for cutthroat trout exposed to chlorine. The geometric mean of these five LC_{50}s was 85 µg/L, with a 95% LCL of the mean LC_{50} of 75 µg/L. The 95% LCL of the mean LC_{50} estimates for brook trout and cutthroat trout were used as input into ICE in order to estimate LC_{50} values for the T&E salmonid species in freshwater.

As described in the Methodology section, EPA (2006) uses the term risk ratio to quantify potentially unacceptable risks to T&E species from exceedance of national acute or chronic water quality criteria. The risk ratio for evaluating the protectiveness of chronic water quality criteria in practical terms is defined as the chronic criterion value divided by the chronic NOEC for a T&E species as shown below, where the T&E species chronic NOEC is either taken from the empirical literature, or is estimated or modeled.

$$R = \frac{C_A \text{ or chronic criterion}}{E_{CA} \text{ or chronic NOEC}}$$

Where: R = Risk ratio
C_A = Assessment exposure concentration (EPA 2006) or chronic water quality criterion
E_{CA} = Assessment effects concentration (EPA 2006) or chronic NOEC

In EPA (2006) the C_A is defined in terms of the exposure concentration allowed by a water quality criterion (i.e. a criterion value itself). In EPA (2006) the E_{CA} is defined as the highest chemical concentration in water (aquatic species) or food (aquatic-dependent species) that would cause an adverse effect to an acceptably-small percentage of a specified species population. In this BE, an E_{CA} is defined as a chronic no effect concentration (chronic NOEC).

The risk ratio approach is the inverse of the hazard quotient (HQ) calculation normally performed in ecological risk assessments (Wenmei et al. 2012), as shown below. In a hazard quotient calculation, a water quality criterion is the assessment exposure concentration (C_A) or toxicity reference value (the denominator) of a hazard quotient, not the numerator as it is in the EPA (2006) risk ratio calculation for water quality criteria.

$$HQ = \frac{\text{EEC}}{\text{TRV or } C_A \text{ (set to equal the chronic water quality criterion)}}$$

Where: HQ = Hazard quotient
EEC = Expected environmental concentration (chemical concentration likely to occur in the environmental media to which organisms are exposed)
TRV = Toxicity reference value (a numerical expression of a chemical's concentration-response relationship with organisms)
C_A = Assessment exposure concentration (EPA 2006). Same definition as used for risk ratio

In ecological risk assessment terms, the EPA (2006) risk ratio for evaluating the protectiveness of water quality criteria to T&E species is a safety factor, not a hazard quotient. The hazard quotient approach is used to describe risks from all other chemicals in this BE. The reason EPA (2006) presents results as risk ratios for chemicals with water quality criteria such as chlorine, and hazard quotients for all chemicals without water quality criteria is so that the interpretation of protectiveness is the same for both chemicals with and without water quality criteria. In both cases, a risk ratio and an HQ < 1 indicates a chemical whose environmental concentration poses acceptable levels of risk. Conversely, a risk ratio and an HQ ≥ 1 is interpreted as a chemical concentration which poses unacceptable levels of ecological risk.

Entering the lower 95% confidence limit (95% LCL), 136 µg/L of the geometric mean 152 µg/L 96-hr LC_{50} for brook trout (Thatcher et al. 1976) into WebICE yielded a predicted bull trout LC_{50} of 114 µg/L, with a 95% lower confidence interval of 45 µg/L (Table ???). Within ICE, the estimated bull trout LC_{50} derived from the empirical brook trout LC_{50} resulted from a family Salmonidae level regression, the only taxonomic comparison ICE was able to perform between bull trout and brook trout (Table ???). When divided by the chlorine ACR of 3.345, the bull trout 95% lower confidence interval of 45 µg/L yielded an assessment effects concentration (EC_A), equivalent to a chronic NOEC, of 13 µg/L. For completeness, Table ??? also shows the results of the bull trout – cutthroat trout ICE regression estimates of the bull trout chlorine LC_{50}. The ICE regressions selected for use to derive the acute LC_{50}s for all T&E species are highlighted in green in Table ???

Using the terminology of the Oregon Toxics BE (Shephard et al. 2008) and the EPA (2006) national guidance for performing Endangered Species Act – Clean Water Act consultations on national EPA water quality criteria, the assessment exposure concentration (C_A, which equals the chronic criterion of 11 µg/L) divided by the assessment effects concentration (EC_A) of 13 µg/L results in a risk ratio of 0.85. A risk ratio less than one indicates that adverse effects are not expected if bull trout are exposed to the chronic chlorine criterion of 11 µg/L. The interspecies correlation estimation line of evidence indicated that the national chronic chlorine criterion is protective of bull trout.

Of the five Salmonidae species whose acute LC_{50}s were estimated using ICE model regressions between the T&E species and the empirical cutthroat trout chlorine LC_{50} (Table ???) from Larson et al. (1978), none of the model predicted EC_As (chronic NOECs) were lower than the freshwater chronic chlorine criterion of 11 µg/L. Results for the risk ratio calculations for protectiveness of the chronic chlorine criteria to all freshwater T&E species that could be quantitatively evaluated are presented in Table ???

Table ???. Risk estimates for the chronic chlorine criterion to T&E fish species in freshwater

Species	ICE estimated LC_{50} (µg/L)	95% LCL of ICE estimated LC_{50} (µg/L)	ACR	Risk ratio	Conclusion
Bull trout	114	44.53	3.345	0.83	Not likely to adversely affect
Chinook salmon	73	56.04	3.345	0.66	Not likely to adversely affect
Chum salmon	73	56.04	3.345	0.66	Not likely to adversely affect
Coho salmon	73	56.04	3.345	0.66	Not likely to adversely affect
Steelhead	74	55.60	3.345	0.66	Not likely to adversely affect
Sockeye salmon	73	56.04	3.345	0.66	Not likely to adversely affect

ICE = Interspecies Correlation Estimation
LCL = Lower Confidence Limit
ACR = Acute-chronic ratio

The conclusion of the Interspecies Correlation Estimation line of evidence for freshwater T&E salmonids is that the freshwater chronic chlorine criterion, which is the Washington hatcheries NPDES permit limit for chlorine discharges to fresh water, is not likely to adversely affect any of the T&E salmonid species in freshwater.

Species Mean Acute Value Line of Evidence

As discussed in the toxicity assessment, a number of 96 hour LC_{50} values exist from studies that do not meet current EPA data quality requirements for use in deriving water quality criteria. Results of these studies are presented in the EPA (1985) *Ambient Water Quality Criteria for*

Chlorine – 1984 document, Tables 1 and 3, and are available for two freshwater T&E species: rainbow trout (steelhead) and coho salmon. Although the rainbow trout and coho salmon studies listed in Table 1 of EPA (1985) were used to derive the chlorine acute water quality criterion, Table ??? in this BE lists the reasons the studies do not meet present day EPA data quality requirements for inclusion in data sets used to derive EPA national water quality criteria. As shown in Table ???, many of the studies not meeting present day data quality requirements were rejected because the dilution water used was chlorinated sewage effluent, a dilution water not considered to be of sufficiently high quality for use in laboratory toxicity tests.

Despite these data quality shortcomings, for the purposes of this BE we have assumed that the rainbow trout and coho salmon acute toxicity studies listed in EPA (1985) do meet present day data quality requirements. This assumption allows us to use empirical acute toxicity data of less than optimal data quality as a secondary line of evidence to derive chronic NOEC estimates using the procedures previously described (i.e. 95% LCL of the empirical LC_{50} divided by the ACR to estimate the chronic NOEC).

The species mean acute 96 hour LC_{50} values and the 95% lower confidence limit of the LC_{50} for coho salmon and rainbow trout were recalculated from the information in Table 1 of EPA (1985) after exclusion of the coho salmon results from Rosenberger (1972) and the rainbow trout results of Merkens (1958). In both cases, these results were excluded because the exposure durations were not 96 hours. The recalculated geometric mean 96 hour LC_{50}s for coho salmon and rainbow trout, and the 95% confidence limits of the geometric mean LC_{50}s were:

Coho salmon – 96 hour LC_{50} = 72 µg/L (65.40 – 79.23 µg/L)
Rainbow trout – 96 hour LC_{50} = 56 µg/L (42.50 – 74.49 µg/L)

Division of the 95% LCL concentrations by the ACR of 3.345 yielded chronic NOEC estimates of 19.55 µg/L and 12.71 µg/L for coho salmon and rainbow trout, respectively. The risk ratios and the conclusions regarding the protectiveness of the freshwater chronic chlorine criterion to coho salmon and steelhead from this secondary line of evidence are as follows:

Coho salmon – Risk ratio = 0.56, not likely to adversely affect
Steelhead – Risk ratio = 0.87, not likely to adversely affect

5.7.4.2.4.3 Chlorine Effects on Prey Species

This section evaluates the potential for adverse effects on T&E species due to direct toxicity to their prey, followed by the loss of food items from the aquatic system. Results are presented in Table 5.??? for the prey of T&E fish species, and are expressed as a range of acute EC_A and chronic EC_A toxicity values for various categories of prey species.

Table 5.??? Toxicity of Chlorine to Food Items of T&E Species

Assessment Exposure Concentrations (C_A): Freshwater Acute = 19 µg/L, Freshwater Chronic = 11 µg/L

Organism Type	Acute EC_A Range (µg/L)	Chronic EC_A Range (µg/L)
Fish	20 – 313	13 - 212
Amphibians	No data	No data
All aquatic invertebrates	5.1 - 1418	3.5 - 957
Aquatic insects	5.1 - 1410	3.5 - 957
Crustaceans	5.9 – 1418	4.0 – 201
Zooplankton	12 – 34	8.3 – 23
Molluscs	31 – 105	21 – 71

No freshwater fish species had acute or chronic EC_A values lower than the respective acute or chronic water quality criteria. This finding supports a conclusion that the chlorine criteria should not have any adverse effect on prey of adult salmonids in freshwater (primarily applicable to bull trout in this BE), which normally feed on fish. The range of chlorine concentrations causing toxicity to invertebrates appears comparable in both fresh and salt water. It also appears evident that at least some crustaceans and insects have a higher tolerance to chlorine than do fish, particularly during short term acute exposures.

As described in the Measures of Ecosystem and Receptor Characteristics section (Section 5.7.4.2.3), juvenile and subadult salmonids feed on a variety of invertebrate species before switching over to the primarily fish diet of adult salmonids. The favored prey appears to be mayflies and dipteran larvae. Table 5.??? indicates that both the lowest calculated acute and chronic EC_A values are lower than the respective acute and chronic chlorine criteria for aquatic insects, crustaceans and zooplankton. Among aquatic insects, data for two of the six available insect species, both of which are mayflies, yielded both acute and chronic EC_A values lower than the respective acute and chronic criteria. A third mayfly species had acute and chronic EC_A values higher than the acute and chronic criteria, as did a caddisfly and two beetle species. Of the remaining 13 invertebrate species with available data (three zooplankton species, three molluscs and seven non-zooplankton crustaceans), only one zooplankton species (*Daphnia magna*, the single most sensitive species to chlorine) and one crustacean (*Gammarus minus*) had calculated acute and chronic EC_A concentrations lower than the respective acute and chronic water quality criteria.

Most aquatic species, including the T&E fish species evaluated in this BE, tend to be opportunistic feeders. Numerous alternative prey species with chronic NOEC or EC_A values above the chlorine criteria exist for T&E fish. This would minimize the potential for adverse effects on T&E fish species from chlorine toxicity to their prey. Therefore, EPA believes that the chronic chlorine criteria will not result in a meaningful reduction in the available prey for T&E fish species.

Risk Characterization of Chlorine in Marine and Estuarine Waters

At the present time, none of the hatcheries in Washington that report the use of chlorine in their operations discharge into estuarine or marine waters. Thus, marine T&E species should not be exposed to chlorine releases from hatcheries. Without exposure to chlorine discharges from hatcheries, there will be no effect of chlorine on the eulachon and three rockfish species currently listed as threatened or endangered in Washington. This conclusion would need to be revisited if one or more hatcheries discharging to marine waters would begin to use chlorine in their operations.

5.7.4.2.4.4 Chlorine Multiple Routes of Exposure Assessment

As discussed in Section 5.7.4.2.1, chlorine is one of the relatively few chemicals that does not require an internally bioaccumulated dose to elicit toxicity to aquatic life. EPA's ECOTOX database contains no information on the bioaccumulation of chlorine, chlorine oxide or chloramines, indirectly supporting the premise that chlorine is an external toxin whose toxicity is elicited externally on the gill surfaces of fish, not an internal toxin. This implies that exposure to waterborne chlorine is the only exposure route of importance to the T&E species under evaluation in this BE. Dietary toxicity from chlorine residues in prey species, or from bioaccumulation of chlorine in the tissues of T&E species are not routes of exposure for chlorine.

5.7.4.2.4.5 Uncertainties Associated with the Chlorine Toxicity Assessment

By design, risk assessments are conservative in the face of uncertainty. In this context, conservative means efforts were made to minimize the chances of underestimating exposure, effects, or risk. The uncertainty analysis portion of this chlorine toxicity assessment is intended to illustrate the degree of confidence in the conclusions of the assessment.

Uncertainty in a risk assessment has four components:

1. **Variation** (e.g. a fish is exposed to a range of chemical concentrations in water, not to a constant concentration of a chemical);
2. **Model uncertainty** (e.g. use of a single species or several target ecological receptors to represent the sensitivity of a T&E species to chlorine introduces uncertainty because of the considerable amount of interspecies variability in sensitivity to a chemical);
3. **Decision rule uncertainty** (e.g. use of a dichotomous decision framework to determine chlorine effects (i.e. NLAA vs. LAA) instead of calculating the probability of an adverse effect at the criteria concentrations); and
4. **True unknowns** (e.g. the toxic effects of chlorine in water on bull trout survival, growth, and reproduction have never been studied, and are unknown).

Consistent with the methods of the problem formulation, receptor-contaminant pairs subject to potentially unacceptable risk from exposure to chlorine in surface waters were identified using conservative methods and assumptions. Examples of conservatism include assumptions that chlorine contaminant concentrations are 100% bioavailable, and assumptions that the most reliable evaluation of chlorine toxicity to a T&E species in the absence of empirical toxicity data for that T&E species comes from basing the assessment on the most closely taxonomically related species to the T&E species of concern that had available and high quality empirical toxicity data.

Not all uncertainties create a conservative bias. Some may lead to underestimation of risk, for example the unavailability of exposure data within the action area. Without empirical data, it may be possible that a spill or other release of chlorine may result in concentrations in localized portions of action area waters near known chlorine dischargers that exceed either the chronic or acute chlorine criteria. As an exposure assumption in this BE is that T&E species are not exposed to concentrations of criteria chemicals higher than the criteria values, a chlorine release could result in this assumption not being met.

The largest single uncertainty in the chlorine toxicity assessment is the absence of high quality measured toxicity data for any of the T&E species evaluated in this BE, with the exception of coho salmon in saltwater. This is a true unknown, and required the use of toxicity data for surrogate species to estimate chlorine effects on T&E species within this BE. Furthermore, the complete absence of empirical toxicity data for any of the T&E rockfish species, eulachon and Oregon spotted frog, as well as for any taxonomically related surrogate species to rockfish, eulachon and Oregon spotted frog made it impossible to employ the ICE model to estimate toxicity for these species from empirical toxicity data of a surrogate species.

Much of the risk characterization is based on the output of ICE models. ICE models are generated from a database of empirical LC_{50} values for a large number of chemicals. To generate an ICE model, all species LC_{50}s are paired with each other by common chemical. Three or more common chemicals per pair are required to develop an ICE model. The more LC_{50} pairs that are available to develop an ICE model, the less uncertain are model predictions and the more statistical power model predictions have (statistical power is the probability that a hypothesis test will correctly reject a null hypothesis that is false). Use of the ICE model to estimate chronic NOEC data from empirical toxicity data of a surrogate species is an example of model uncertainty in risk assessment.

Uncertainty in the ICE models is described by the percent cross-validation success statistic. According to Raimondo et al. (2013), the percent cross-validation success rate for each model is the proportion of data points that are predicted within 5-fold of the actual LC_{50} value. There is a strong relationship between taxonomic distance and cross-validation success rate, with uncertainty increasing with larger taxonomic distance. For fish and aquatic invertebrates, ICE models overall predict within 5-fold and 10-fold of the actual LC_{50} value with 91 and 96% certainty for surrogate and predicted taxa within the same family, and for 86 and 96% within the same order. All ICE models used in the chlorine toxicity assessment had cross-validation success rates greater than 86%, which would be the minimum acceptable cross validation percentage for any ICE model run between two species of the order Salmoniformes. Current fish taxonomy (Eschmeyer 1998) recognizes the family Salmonidae as the only family with currently living species within the order Salmoniformes.

EPA's aquatic life criteria are designed to protect 95% of aquatic genera from adverse effects, not 100% of aquatic species. Given this design, it is possible that one or more important prey species of T&E species within the action area not tested may be subject to toxic effects at chlorine concentrations lower than the chronic criteria. Loss of such species could reduce the prey base available to juvenile and subadult age classes of T&E species. Four of 19 invertebrate species (21%) for which empirical chlorine toxicity data are available were affected by TRC concentrations lower than the chronic criteria.

Use of acute-chronic ratios to convert 96-hr LC_{50} data to chronic NOECs or maximum acceptable toxicant concentrations (MATC's) introduces uncertainties into the evaluation of the chronic criteria, as the empirical data from which the geometric mean 3.345 ACR in EPA (1985) was derived differ for the three species ACR's used to calculate the geometric mean ACR. An ACR of 3.345 is low compared to the ACR of most other chemicals. A study by Raimondo et al. (2007) determined a geometric mean acute-chronic ratio of 8.3 from a data set of 456 same-species pairs of acute and maximum acceptable toxicant concentrations for metals, narcotics, pesticides, and other organic chemicals. The chlorine ACR of 3.345 may be indicative of a chemical with a relatively steep dose response curve, meaning the difference between adverse and no adverse effect concentrations for a given species may be small. Steep dose-response curves for chlorine have been empirically identified for fish species (Tsai et al. 1990).

Although not as much an issue with chlorine compared to other chemicals used at Washington fish hatcheries and evaluated in this BE, the limited quantity of stream discharge data in the vicinity of most Washington hatcheries, combined with the complete absence of any water quality monitoring for hatchery chemicals in receiving waters where T&E fish are located renders any estimate of environmental concentrations to which T&E species are exposed a true unknown in this BE. Limited information is available from some hatcheries that permits estimates of hatchery chemical concentrations at the 'end of pipe' where hatchery discharges enter receiving waters. But these 'end of pipe' estimates are an example of an uncertainty that overestimates risk to T&E species in the environment, albeit by an unknown amount.

5.7.4.2.5 Chlorine Effects Determinations and Summary

The conclusions of this BE for the T&E species where chlorine risks could be quantified are as follows:

Freshwater:
Bull trout – not likely to adversely affect
Chinook salmon – not likely to adversely affect
Chum salmon – not likely to adversely affect
Coho salmon – not likely to adversely affect
Steelhead – not likely to adversely affect
Sockeye salmon – not likely to adversely affect

Toxicological information is not available which permits risks to be quantified to the remaining fully aquatic T&E species in Washington.

Evaluation of chlorine toxicity to other fish species, some of which are potential prey species of the T&E salmonids that could be quantitatively evaluated in this BE, indicated that both the acute and chronic criteria are protective of all fish species for which empirical toxicity data are available. Evaluation of chlorine toxicity to invertebrate species indicates that although adverse effects have been observed on several invertebrate species at chlorine concentrations lower than the acute and chronic criteria, numerous alternative prey species exist for salmonids with acute and chronic EC_A values above the chlorine criteria.

Bioaccumulation risks and dietary ingestion risks from chlorine are unlikely, based on the no to low likelihood of chlorine bioaccumulation in either T&E species or their prey. This is true for both fully aquatic and aquatic-dependent species.

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